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Evaluation of *Cymbopogon schoenanthus* essential oil in lambs experimentally infected with *Haemonchus contortus*

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ABSTRACT

Hematophagous gastrointestinal parasites cause significant economic losses in small ruminant grazing systems. The growing reports of multi-drug resistant parasites call for intensive research on alternative treatments for anthelmintics to help small ruminants cope with these parasites. Two-month-old lambs with mean body weight (BW) of 22.5 kg were experimentally infected with a multidrug-resistant *Haemonchus contortus* strain. Infected animals were dosed orally with *Cymbopogon schoenanthus* essential oil to evaluate its anthelmintic potential. Eighteen animals were allocated into three groups of six animals, and each received one of the following treatments: Group 1 – control (10 mL of water), Group 2 – *C. schoenanthus* essential oil (180 mg/kg BW); and Group 3 – *C. schoenanthus* essential oil (360 mg/kg BW). Animals received the oil once a day for 3 consecutive days. Lambs were evaluated clinically for blood biochemistry before, at 1, 5, 10, 15 and 20 days after treatment, and then were euthanized to assess the total worm burden. No statistically significant reduction in fecal egg count, packed cell volume or total worm count was observed after treatments. Also, no statistical difference among group means for blood levels of urea, creatinine, albumin, alkaline phosphatase, aspartate aminotransferase and gamma glutamyl transferase was found. Larval development assay (LDA) and egg hatch assay (EHA) were performed from feces of treated animals at 1, 5, 10 and 15 days after essential oil administration. An inhibition in LDA was observed 1 day after the 3-day treatment in larvae from feces of animals treated with 360 mg/kg essential oil. In conclusion, the essential oil at the doses of 180 mg/kg and 360 mg/kg was safe to sheep, but failed as an anthelmintic treatment when applied to young sheep artificially infected with a multidrug-resistant *H. contortus* strain.

1. Introduction

Haemonchus contortus is the most important hematophagous gastrointestinal nematode (GIN), representing a major health and economic obstacle for the feasibility of grazing sheep production systems in the tropics. Hot and humid conditions favor larval development, increasing the reinfection rates of animals grazing in contaminated pastures. The severe blood loss caused

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by this parasite can result in anemia, anorexia, reduction in body weight, depression, and death. This scenario is aggravated by the multi-drug resistance of *H. contortus* to several commercial anthelmintics (Rowe et al., 2008).

Alternative methods and anthelmintic products to control *H. contortus* are urgently needed for the feasibility of small ruminant production systems. Regarding alternative anthelmintic products, several researchers have tested plant-derived products, including essential oils. For instance, Valencia orange essential oil (Squires et al., 2010) and *Eucalyptus staigeriana* essential oil (Macedo et al., 2010) lead to successful reduction in fecal egg count and parasite burden in sheep and goats, respectively, infected with *H. contortus*.

Cymbopogon schoenanthus belongs to the family Poaceae (Gramineae) and is reported to have sedative, digestive and aromatic properties, with a strong and characteristic aroma, with some reports on its insecticidal activity (Ketoh et al., 2002; Koba et al., 2007). Although we found no studies on the toxicity of *C. schoenanthus* essential oil, it is known that geraniol, the main constituent (59%) from *C. schoenanthus* has an oral LD_{50} in rats of 3600 mg/kg, and geranial (13%), the second main constituent has an oral LD_{50} in rats of 4960 mg/kg (Material Safety Data Sheet, Sigma–Aldrich, 2011).

A previous study (Katiki et al., 2011) demosntrated the *in vitro* anthelmintic activity of *Cymbopogon schoenanthus* essential oil against ovine trichostrongylids through the egg hatch, larval development, larval feeding, and larval exsheathment assays.

The objectives of this work were to evaluate the *in vivo* and *in vitro* anthelmintic activities of *C. schoenanthus* essential oil in lambs infected with a drug-resistant *H. contortus* strain in lambs. *In vivo* parameters included the fecal egg count (FEC), total worm count (TWC), packed cell volume (PCV), and possible toxicity evaluated by changes in the profile of liver and kidney enzymes of lambs treated with the essential oil. *In vitro* parameters included inhibition of both egg hatch and the larval development of nematodes collected from lambs experimentally infected and treated orally with 180 and 360 mg of essential oil per kg of live body weight.

2. Materials and methods

2.1. Animals

Eighteen Santa Ines male lambs, approximately 2 months old, and with mean live body weight (BW) of 22.5 kg were kept indoors in collective stalls, where they were fed Coastcross hay (*Cynodon dactylon*), mineral salt, and water "*ad libitum*". All experimental protocols were approved by the FMVZ-UNESP-Botucatu Animal Care and Use Committee.

2.2. Parasite cleansing pre-experiment

Animals received levamisole phosphate (Ripercol F®, Fort Dodge, Brazil) and albendazole (Valbazen®, Pfizer, Brazil) at double prescription dose every 24 h during 3 consecutive days to treat natural infections by nematodes (Amarante et al., 2004). After treatment, fecal samples were collected directly from the rectum of each animal to confirm their worm-free status.

2.3. Artificial infection

One donor sheep was maintained with a single strain of *H. contortus* that was multidrug-resistant (levamizole, albendazole, ivermectin, moxidectin, closantel and triclorfon), as described by Almeida and collaborators (2010). Fecal cultures were done to obtain infective larvae (L_3). Each lamb was infected orally with 4000 L_3 of this *H. contortus* strain.

2.4. Groups

Twenty-six days after infection, sheep were weighed and nematode fecal egg counts (FEC) were recorded for each animal. Lambs were allocated into experimental groups based on their FEC (highest to lowest). Animals were allocated to groups with six animals each. The groups were: Group 1 – control (10 ml of water); Group 2 – treated with *C. schoenanthus* essential oil (180 mg/kg body weight (BW)); and Group 3 – treated with *C. schoenanthus* essential oil (360 mg/kg BW). These doses were chosen after a pilot experiment where some efficacy, and no toxicity, was observed. Animals were fasted for 8 h pre-treatment and 4 h post-treatment. To make sure lambs received correct volume of essential oil, the volume of oil was pumped first into a graduated syringe, and water was added to a total volume of 10 ml. This water volume was sufficient to carry the oil into the rumen of the animal without losses. This dosing procedure was done three times per treated animal in intervals of 24 h.

2.5. Essential oil chromatographic analysis

C. schoenanthus oil was purchased from WNF Ind. & Com. Ltda (R. Dr. Mario Pinto Serva, 64, Sao Paulo-SP, Brazil), Lot no. 10608, $d = 0.911$.

The oil was analyzed by gas chromatography (GC) coupled to a mass spectrometry (MSD) and flame ionization (FID) detectors (Agilent GC System 6890 Series for the Mass Selective Detector, and Agilent 5973 Network for the FID detector). Each sample was analyzed in two separate columns (HP-5, 30 m, 0.25 mm ID, 0.25 μm film), attached to either the MSD or the FID. The conditions for both inlets and columns were the same with helium as a gas carrier, at a constant flow of 1 mL/min. Inlet temperature was 220 °C, with a temperature program of 60 °C for 1 min, increasing 4 °C/min to reach 200 °C in 15 min. The temperature for the FID was set at 220 °C and for MSD at 280 °C. Qualitative analysis was based on a comparison of retention times and indexes on both columns and mass spectra with corresponding data in the literature and mass spectral libraries (Wiley 275).

2.6. Pre- and post-treatment clinical evaluation

Feces were collected directly from the rectum to determine the FEC before, 1, 5, 10, 15, and 20 days after